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Influence of microwave frequency electromagnetic radiation on terpene emission and content in aromatic plants

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Abstract

Influence of environmental stress factors on both crop and wild plants of nutritional value is an important research topic. The past research has focused on rising temperatures, drought, soil salinity and toxicity, but the potential effects of increased environmental contamination by human-generated electromagnetic radiation on plants have little been studied. Here we studied the influence of microwave irradiation at bands corresponding to wireless router (WLAN) and mobile devices (GSM) on leaf anatomy, essential oil content and volatile emissions in *Petroselinum crispum*, *Apium graveolens* and *Anethum graveolens*. Microwave irradiation resulted in thinner cell walls, smaller chloroplasts and mitochondria, and enhanced emissions of volatile compounds, in particular, monoterpenes and green leaf volatiles. These effects were stronger for WLAN-frequency microwaves. Essential oil content was enhanced by GSM-frequency microwaves, but the effect of WLAN-frequency microwaves was inhibitory. There was a direct relationship between microwave-induced structural and chemical modifications of the three plant species studied. These data collectively demonstrate that human-generated microwave pollution can potentially constitute a stress to the plants.

Keywords

Microwave; abiotic stress; essential oils; volatile organic compounds; aromatic plants

INTRODUCTION

Aromatic plants represent an important resource for human nutrition, due to their valuable properties, including medicinal benefits (Bonjar, 2004; Wong and Kitts, 2006; Bakkali et al., 2008; Ortan et al., 2009; Cornara et al., 2009). Therefore, understanding their chemical composition and how the properties of aromatic plants are affected by key climate change factors as well as human-generated pollution are research topics of major interest.

The key property of aromatic plants is the presence of essential oils that play important roles in plants acting as direct defenses against pathogen and herbivore attacks (Rhoades, 1977;

Lewinsohn, 1991; Fugmann et al., 1997; Reddy et al., 2001). The essential oils are very complex natural mixtures that consist of molecules produced through different secondary metabolic pathways, characteristically containing terpenoids, benzenoids and sometimes aliphatic compounds (Bauer et al., 1998; Eggersdorfer, 1998; Cheng et al., 2007; Bakkali et al., 2008).

Both the composition and content of essential oils has been shown to strongly depend on plant species and environmental conditions (Langlille and MacLean, 1976; Letchamo and Gosselin, 1996; Zabaras et al., 2002; Manzan et al., 2003). These aspects are relevant because plants in natural conditions as well as in agricultural fields are exposed to a plethora of abiotic and biotic stresses and the importance of several biological and environmental stresses is expected to increase in the future (Peñuelas and Estiarte, 1998; Lobell, 2008; Craufurd and Wheeler, 2009; Jacob and Winner, 2009; Niinemets, 2010b).

The key abiotic stresses(Lobell, 2008; Craufurd and Wheeler, 2009; Jacob and Winner, 2009) of contemporary economical importance for plant growth worldwide are drought, heat, cold (chilling and freezing), high salinity, soil mineral deficiency and toxicity. Furthermore, diffuse environmental pollution, including air and soil pollution constitutes a major problem for agriculture and human health (Gauderman et al., 2004; WHO, 2004; Copaciu et al., 2013; Opriş et al., 2013). It was demonstrated that the blend of volatile organic compounds emitted by aromatic plants under stress factors is complex (Rodrigues-Navas et al., 2012). The complexity of volatile emissions in species having specialized storage structures for volatiles results from the circumstance that there may be emissions directly coming from storage and de novo emissions independent of storage (Staudt et al., 1997; Niinemets et al., 2010b; Monson et al., 2012; Grote et al., 2013; Li and Sharkey, 2013).

Among the novel potential pollution sources is the enhanced use of mobile phones and wireless devices generating an exponentially increased level of electromagnetic radiation in the microwave range of radiation frequencies (1-100 GHz). There have been some studies on microwave effects on plants showing no significant effects, while others have demonstrated important modifications in plant functioning. Laboratory growth experiments in plants subject to magnetic fields demonstrated that plants were taller and heavier (Martínez et al., 2003). Likewise, germination of *Cicer arietinum* L. seeds and early development were enhanced upon exposure to a moderate magnetic field (Vashisth and Nagarajan, 2008). It has been shown that electromagnetic radiation at broadcast-frequency (0.2-30 MHz) altered the cellular contents of calcium and sulfur, effect associated with the power of radiation (Balmori Martínez, 2003), while in animal cells has been observed that microwaves (frequency of 147 MHz, amplitude-modulated at 16 Hz) can influence the intercellular communication through altering the functioning of the calcium channels (Balmori Martínez, 2003). Exposure to microwaves (frequency of 9.75 GHz and low intensity) of wheat (*Triticum aestivum*) plants has resulted in cytogenetic changes (Pavel et al., 1998; Balmori Martínez, 2003). Studies have also shown alterations in condensed chromatin distribution of meristem cells exposed to low magnetic fields (Belyavskaya, 2001; Belyavskaya, 2004). In general, these studies collectively suggest that the effects of electromagnetic fields on plants can be variable.

It is, however, unclear what the mechanism of low-energy microwave irradiation effects on plant is. While high energy microwave-radiation can break the chemical bonds (Caldwell et al., 1995; Barnes and Cardoso-Vilhena, 1996), the quantum energy of microwave radiation is low and mainly can have thermal effects, heating up selectively plant structures and possibly also alter the conformation of biomolecules, such as proteins, nucleic acids and membrane lipids. Furthermore, modifications in biomolecular tertiary structure can importantly alter the rate of physiological processes, again implying that microwaves can lead to stress conditions in plants (Takeuchi and Thornber, 1994; Ha et al., 1997; Havaux, 1998). Thus, it is important to gain more conclusive insight into the effects of microwaves on plant performance.

The aim of the present study was to investigate the influence of microwave irradiation on the ultrastructure of leaves, the essential oil content and volatile organic compounds emission of three aromatic plant species of the Apiaceae family, parsley (*Petroselinum crispum* L.), dill (*Anethum graveolens* L. subsp. *hortorum* Alef.) and celery (*Apium graveolens* L.). The stress application consisted in three weeks microwave irradiation of plants at bands corresponding to wireless router (WLAN) and mobile devices (GSM). As the emissions of stress volatiles such as green leaf volatiles (GLV) and specific terpenes are enhanced upon exposure to different stresses (Heiden et al., 2003; Beauchamp et al., 2005; Copolovici et al., 2011; Niinemets et al., 2013), we hypothesized that microwave irradiation leads to enhanced emission of stress volatiles. In addition, we intend to investigate how microwave irradiation affects the leaf structure, content and composition of essential oils in these aromatic plants of nutritional and medicinal importance.

MATERIALS AND METHODS

Plant material and growth conditions

Plant material including parsley (*Petroselinum crispum* cv. Plain leaved 2) (P), dill (*Anethum graveolens subsp. hortorum* cv. Common) (D) and celery (*Apium graveolens* cv. Pascal Giant) (C) were grown in laboratory from seeds obtained from Agrosel (Câmpia Turzii, Romania). Fifteen seeds were sown in 150 mL plastic pots (height x diameter of 8.5 × 6.5 cm) filled with commercial garden soil.

Three weeks after seeding, the vessels with plants were placed in three identical anechoic chambers (Surducan et al., 2012) characterized by a degree of isolation of 60 dB at radio-frequency range between the exterior and interior. The fully-closed chambers were maintained under the same conditions of light intensity at 300 µmol m^{-2} s^{-1} (provided from four 4W MR16 LED lamps (every lamp consisting of 26 warm white SMD 5050 LED at 3300K), temperature (25°C), CO_2 concentration (385 ± 20 ppmv) and humidity (65%). One chamber was for non-treated control plants, while plants in the other two chambers were subjected to microwave irradiation. The microwave irradiation was performed at bands corresponding to mobile devices (GSM) using a modified AP5200 generator (D-LINK, China), operating in four bands (860 – 910 MHz frequency range, Pout 29 dBm), and to wireless router (WLAN) using a D-LINK wireless router 802.11g/2.4 GHz (2.412 – 2.48 GHz frequency range, Pout 19 dBm). In the irradiation chamber there is one stick antenna placed in the center of the ceiling. The exposure levels where chosen in agreement with the microwave irradiation levels measured in open space for heavily used GSM networks ($100mW/m^2$) and for indoor WLAN ($70mW/m^2$) communication protocols. The power density to the base of chambers was measured with a spectrum analyzer SPECTRAN HF 4060, AARONIA AG (Germany). Both control and microwave-irradiated plants were watered every 2 days with 10 mL of bidistilled water (bidistillator AcquaMatic model AWC/4D, Hamilton Laboratory Glass Ltd., Kent, UK).

Irradiation was performed during three weeks, after which plants were removed from the chambers for measurements of volatile organic compound (VOC) emission and analyses of leaf structure and essential oil content. All measurements of volatile organic compound (VOC) emission and analyses of leaf structure and essential oil content have been replicated with eight different plants.

Transmission electron microscopy measurements

Samples for transmission electron microscopy (TEM) were contrasted with 2% uranyl acetate in 50% ethanol solution for 2 min and in 0.2% lead citrate in 0.1 M sodium hydroxide solution for 2 min. The samples were dehydrated in ethanol series and embedded in epoxy resin, Epon 812. The samples were cut in an Ultramicrotome, Leica UC6 with a diamond knife and the ultrathin samples (100 nm) were analyzed with a 120kV TEM Model JEM 1010 (Jeol USA Inc., Peabody, MA, USA). The number of chloroplasts, mitochondria, starch grains in the chloroplasts and nuclei were determined in the palisade mesophyll cells for replicate plants under each treatment.

Essential oil extraction

Samples of fresh plant material of 1 g were frozen in liquid nitrogen, pulverized and essential oils were extracted with 2 mL of 1:1 (v/v) mixture of HPLC-grade diethyl ether and *n*-hexane (Merck, Germany). For extraction, plant material was initially soaked for 10 minutes with the solvent mixture, and then extracted in an ultrasonic bath (Elmasonic S 15H, 37 kHz) for 30 minutes at 30°C. Each extraction was performed using five parallel samples. In all cases, extracts were decanted and filtered through nylon syringe filters (0.45 μm) before use.

Volatile organic compounds (VOC’s) sampling and photosynthetic parameters determinations

VOC sampling was performed using a portable gas exchange system GFS-3000 (Waltz GmbH, Effeltrich, Germany). The system has an environment-controlled cuvette with 8 cm^2 window area and multiple leaves were enclosed in the cuvette to fill the whole cuvette window. A volume of 4 L of air exiting the cuvette was sampled in a multibed stainless steel cartridge (8.88 × 0.65 cm, Supelco, Bellefonte, PA, USA) filled with Carbopack adsorbents (C 20/40 mesh, C 40/60 mesh, and X 20/40 mesh). The chamber air was sampled at a flow rate of 200 mL min^{-1} for 20 min using a 1003-SKC constant flow sampling pump (SKC Inc., Houston, TX, USA) at room temperature. Background air samples were taken before and after the measurements using the same system without the leaves enclosed in the cuvette. Using the same system, CO_2 and H_2O concentrations have been measured. The rates of net assimilation (A) and stomatal conductance to water vapour (g_s) were calculated per unit enclosed plant leaf area according to von Caemmerer and Farquhar (1981).

Essential oils and volatile organic compounds analysis

For both essential oils and VOC analysis, a Shimadzu QP2010 Plus gas chromatograph coupled with quadrupole mass spectrometer (GC–MS) (Kyoto, Japan) was used. The conditions for essential oils analysis were as follows: injector temperature was 215°C, initial oven temperature at 40°C was held for 1 min; ramped at 5°C min^{-1} up to 200°C, held at this temperature for 1 min; ramped at 10°C min^{-1} up to 220°C and held for further 5 min. Helium (purity 99.9999 %, Elmer Messer Gaas AS, Tallinn, Estonia) was employed as carrier gas with a constant flow rate of 1 mL min^{-1}. The mass spectrometer was operated in electron-impact mode (EI) at 70 eV, in the scan range m/z 30 – 400, the transfer line temperature was set at 240°C and ion-source temperature at 150°C.

For VOC analysis, an automated cartridge desorber Shimadzu TD20 (Kyoto, Japan) was used. The volatiles were analyzed according to the method described in detail in Copolovici *et al*., 2009.

The essential oils and volatile organic compounds were identified by comparing the mass spectra of individual compounds with the spectra of GC purity external standards (Sigma Aldrich, St. Louis, MO, USA) and with the spectra of NIST Library.

Statistical analysis and data handling—For transmission electron microscopy (TEM) analyses, five replicate measurements, and for essential oils and volatile organic compound three replicates with independent samples of plants were available and we report means of the replications ± SE at each treatment. The means were statistically compared with Student ANOVAs followed by post hoc Tukey's tests using ORIGIN 8 (OriginLab Corporation, Northampton, MA, USA). All statistical differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Ultrastructural analyses and photosynthetic parameters

Irradiation resulted in both qualitative and quantitative modifications in leaf anatomy. Palisade and spongy-parenchyma cells exhibit slightly wavy walls in leaves of irradiated plants, while in leaves of control plants were straight-walled (Fig. 1).

This indicates alterations in spatial arrangement of cells in leaf lamina cross-section and a moderate decrease of lamina turgidity. In fact, cell wall thickness was reduced by microwave-irradiation, and generally more strongly by the treatment with WLAN-frequency microwaves (Table 1).

Chloroplasts retained their ultrastructure and normal arrangement within cells, but chloroplasts tended to be smaller in irradiated leaves, especially in the case of WLAN frequency microwaves (Table 1). The ratio of starch grain area to chloroplast area was somewhat increased in treatment with GSM-frequency microwaves in *P. crispum* and *A. graveolens*, while it was reduced in WLAN-frequency microwave treatment in *P. crispum*

(Table 1). Nevertheless, the differences among the treatments were relatively small, indicating moderate effects of microwave treatments on starch accumulation.

The mitochondrion length generally decreased in microwave-treated plants, especially in the case of plants irradiated with WLAN-frequency microwaves (Table 1). In addition, the number of mitochondrial cristae was also somewhat less, suggesting a certain decrease in their metabolic activity. Nuclei of most cells showed normal structure, however abundance of heterochromatin and presence of wavy contours tended to be greater in microwave-treated plants.

The anatomical modifications were qualitatively similar among species (Table 1). In all species, the treatment with WLAN-frequency microwaves resulted in greater anatomical changes than the treatment with GSM-frequency microwaves (Table 1). However, the microwave-induced changes were the strongest in *Anethum graveolens*, where all treatments differed from each other (Table 1) followed by *P. crispum* and *Apium graveolens*. In this species, chloroplast length did not differ among the treatments and mitochondrion length in GSM-frequency microwaves was not different from that in control plants (Table 1).

These data collectively demonstrate important alterations in foliage ultrastructure by microwave irradiation, and are in agreement with observations in wheat (*Triticum aestivum*) exhibiting pronounced cytogenetic changes in response to microwaves (Pavel et al., 1998; Balmori Martínez, 2003). It was shown that under the influence of low-intensity microwaves in the species of *Triticum aestivum*, as compared to the control plants different types of chromosomal aberrations appeared: delayed chromosomes, micronuclei, interchromosomal bridges, chromosomal fragments (Pavel et al., 1998). In meristem cells of *Pisum sativum* L. roots exposed to low-magnetic field were observed ultrastructural changes such as a noticeable accumulation of lipid bodies, development of a lytic compartment (vacuoles, cytosegresomes and paramural bodies), and reduction of phytoferritin in plastids. The most sensitive organelle to low-magnetic field application was mitochondria, whose size and relative volume in cells increased, matrix was electron-transparent, and cristae reduced (Belyavskaya, 2001).

Photosynthesis parameters (assimilation rates and stomata conductance to water vapor) have been affected by microwaves exposure (Figure 2). Even more, both parameters are influenced by the strength of the stress.

Overall, reduction in the size of organelles may indicate that photosynthesis and respiratory metabolism was somewhat impaired by microwave treatment (Louwerse and van der Zweerde, 1977; Lichtenthaler, 1981; Griffin et al., 2001; Terashima et al., 2011). On the other hand, reduction in cell wall thickness reduces mesophyll diffusion conductance to chloroplasts thereby potentially compensating for reduced physiological potentials and altered stomatal conductance to water vapor (Terashima et al., 2006; Tosens et al., 2012a; Tosens et al., 2012b; Tomás et al., 2013). However, reduced cell wall thickness reduces cellular resistance to low leaf water potentials (Niinemets, 2001). Thus, microwave irradiance may importantly decrease plant drought resistance.

Changes in essential oils content in response to microwave irradiation: general patterns

Many aromatic plants have specialized structures for the storage of volatiles and the composition of essential oils in these storage structures is often complex (Letchamo et al., 1995; Manzan et al. 2003; Rajabi et al., 2013). Our study also observed complex composition of essential oils in the studied species, 10 compounds were detected in *P. crispum*, 11 compounds in *Anethum graveolens* and 7 compounds in *Apium graveolens*. In all species, monoterpenes constituted a significant component of the essential oil (Fig. 3). In addition, several specific benzenoids were also dominating components of the oil: apiol in *P. crispum*, and myristicin and dillapiole in *Anethum graveolens* (Fig. 3). Lipoxygenase pathway compounds were important constituents in essential oil in *P. crispum* and *Apium graveolens* (Fig. 3). In addition, the main volatile compounds in *Apium* graveolens are 3-hexen-1-ol, myrcene, α-ocimene, γ-terpinene (Fig. 3). These results broadly agree with past observations of essential oils produced by these species (Deng et al., 2003; Orav et al., 2003).

Multiple environmental factors have been shown to modify the content of essential oil (Langlille and MacLean, 1976; Gershenzon, 1984; Letchamo et al., 1994; Wannaz et al., 2003; Peñuelas et al., 2011). Such modifications in essential oil content have been often explained on the basis of hypotheses linking growth, and primary and secondary metabolism (Bryant et al., 1983; Herms and Mattson, 1992; Peñuelas and Estiarte, 1998). According to these hypotheses, when sink activity (growth) rate decreases and carbon dioxide availability is in excess of that naturally occurring in the atmosphere, plants increase the rate of synthesis of secondary metabolites such as essential oils (Bryant et al., 1983; Herms and Mattson, 1992; Peñuelas and Estiarte, 1998). In agreement with this hypothesis, water deficit has been shown to increase the yield of essential oil and affected its relative composition in *P. crispum* (Petropoulos, 2008). Analogously, enhanced salinity increased the essential oil yield for *Anethum graveolens* plants under stress (Ghassemi-Golezani et al., 2011).

In our study, microwave irradiation by GSM-frequency microwaves generally increased the essential oil contents (Fig. 3), while the effect of WLAN-frequency microwaves was less clear, varying from positive or negative for different compounds and species (Fig. 3). In a like manner, ozone stress induced two distinct pathways in *P. crispum* (Eckey-Kaltenbach et al., 1994) suggesting that different types and severity of stress can lead to qualitatively different responses. Taken together, these results are in partial agreement with several past observations indicating enhanced production of essential oils under stress and also are in agreement with the evidence of impaired photosynthetic and respiratory metabolism under microwave irradiation (Table 1). However, differently from essential oils, the anatomical modifications were significant under WLAN microwave irradiation (cf. Table 1 and Fig. 1c).

Species and microwave-frequency effects on essential oils

Although the effects were broadly similar among species, important species differences were observed in individual compound responses to GSM- and WLAN-frequency microwaves. Among the three plant species tested in these experiments, the strongest effects of microwave irradiation on essential oils were observed on *Anethum graveolens* plants (Fig. 3).

For individual compounds in *P. crispum*, the microwave irradiation produced by GSM generator statistically increased 3-hexen-1-ol, myrcene, α-phellandrene, β-phellandrene, myristicin and apiole contents. Compared to the reference, the strongest increase in response to GSM-frequency irradiation was observed for apiole (more than seven times greater content, Fig. 3a). The WLAN-frequency microwaves statistically increased the content of α-pinene, β-phellandrene, myristicin and apiol in this species (Fig. 3a).

In *Anethum graveolens* irradiated with GSM microwaves, increased content was observed for β-pinene, α-phellandrene and dillapiole (Fig. 3b). However, WLAN-frequency microwaves reduced α-phellandrene, myristicin and dillapiole content, whereas the greatest reduction was observed for myristicin (approximately to the level 18% of that in reference plants, Fig. 3b).

In *Apium graveolens*, both types of microwaves used in this study increased 3-hexen-1-ol content (Fig. 3c). Irradiation by WLAN-frequency microwaves reduced myrcene (19%) and α-ocimene (21%) contents (Fig. 3c).

Species-differences in environmental responses to stress factors have been demonstrated (El-Keltawi and Croteau, 1986; Mangas et al., 2006) although interspecific studies have been rare. Species ranking according to anatomical modifications was similar to the ranking based on essential oil changes (cf. Table 1 and Fig. 3). The structure of *Apium graveolens* leaves was the least affected by microwave irradiation and the effect on leaf chemistry was also the least in this species.

General patterns in volatile organic compounds emissions

Our data demonstrate that the emissions observed did reflect a mixture of both storage emission consisting of compounds present in essential oils and de novo emissions. The blend of volatiles was very complex and, in all plant species, the non-stressed plants also emitted monoterpenes and benzenoids present in essential oils, in some cases even compounds not-present in essential oils (Fig. 4). The number of compounds detected in the emissions was greater than in the essential oils, and characteristic de novo released stress volatiles were observed (Fig. 4). 16 compounds were detected in the emissions of *P. crispum*, 16 compounds in *Anethum graveolens* and 20 compounds in *Apium graveolens.*

There was evidence of similar enhancement of essential oils and emissions for several monoterpenes, especially for GSM microwave treatments in *P. crispum* and *Anethum graveolens* (cf. Figs. 3 and 4). However, in these species, emissions were more strongly enhanced under WLAN microwave treatment, which appeared to have an inhibitory effect on the content of the same terpenoids, e.g. α-pinene and β-phellandrene (cf. Figs. 3 and 4). Although there was evidence of parallel changes in contents and emissions for some volatiles in species, and for some treatments, this evidence suggests that the storage and de novo emissions cannot be fully teased apart in the current study. Nevertheless, the data suggest that the total emissions and especially treatment differences reflect to a large degree the microwave-induced de novo synthesized plant volatiles.

Compound-class, species- and treatment-specific differences in volatile emissions

Among the de novo emissions, green leaf volatiles (GLV), also called volatiles of lipoxygenase pathway (LOX volatiles) are released in plants in response to different stresses (Copolovici and Niinemets, 2010; Copolovici et al., 2011; Copolovici et al., 2012). GLVs are formed in the hydroperoxide lyase pathway of oxylipin metabolism from free octadecanoic fatty acids and consist usually of a mixture of C6 aldehydes and ketones (Matsui, 2006). In our study, all microwave-irradiated plants emitted the following GLVs: (*E*)-2-hexenal, (*Z*)-3-hexenol, 1-hexanol, while the emissions of GLVs were very low at the level of detection limit of our device in control plants (Fig. 4).

In general, in all plant species studied, the emissions of GLV were greater for WLAN-frequency microwaves compared to GSM-frequency microwaves (Fig. 4, $P < 0.001$ for all). These results suggest greater stress in the case of WLAN microwave irradiation, and are in agreement with the more significant changes in anatomy of leaves induced by WLAN microwaves (Table 1). Stronger GLV emissions under more severe stress have been shown for water (Capitani et al., 2009), ozone (Beauchamp et al., 2005), herbivory attack (Allmann and Baldwin, 2010) and temperature (Copolovici et al., 2012) stresses.

The GLV emissions of the *P. crispum* and *Anethum graveolens* were dominated by the 1-hexanol (Fig. 4), while in *Apium graveolens* the main component was (*Z*)-3-hexenol that was also important constituent in the essential oil in this species (Figs. 3 and 4). The total GLV emission from *P. crispum* and *Anethum graveolens* was five times higher than from *Apium graveolens*. As with the essential oil content (Fig. 3), *Apium graveolens* was clearly less sensitive to the microwave fields than *P. crispum* and *Anethum graveolens*.

The monoterpenes detected in the emissions were α-pinene, β-pinene, camphene, limonene, 3-carene, *para*-cymene, β-phellandrene, (*E*)-β-ocimene, eucalyptol and bornyl acetate. In *P. crispum*, emission of α-pinene, β-pinene and β-phellandrene were dominant and enhanced by microwave irradiation, especially in the case of WLAN-frequency microwave treatment (Fig. 4a). Treatment effects on monoterpene emissions were similar for *Apium graveolens* and *Anethum graveolens*, but the main components are at some extent different (Figure 4b and 4c). Monoterpene emissions from *Anethum graveolens* were dominated by α-pinene, α-phellandrene and limonene, and these emissions were enhanced by microwave irradiation (Fig. 4b). In *Apium graveolens*, the emissions were almost four times lower than in the other species and were dominated by α-pinene, β-pinene and limonene (Fig. 4c). The emission of terpenes was inhibited by microwave irradiation similarly to the content of essential oils (Figs. 3 and 4).

Overall, these emitted monoterpenes are characteristic plant-released compounds and are not specific to stress-induced emissions (Staudt et al., 1997; Kesselmeier and Staudt, 1999; Staudt et al., 2000; Niinemets et al., 2010b). However, the emission rates of these typical monoterpenes is also often enhanced in stress conditions (Vuorinen et al., 2004; Blande et al., 2007; Heijari et al., 2008; Copolovici et al., 2011; Copolovici et al., 2012), implying that induced and constitutive emission are often difficult to separate. Among the characteristic induced monoterpenes (Staudt and Bertin, 1998; Hakola et al., 2001; Noe et al., 2006; Niinemets et al., 2010b), emissions of (*E*)-β-ocimene and 1,8-cineole were strongly enhanced by microwave-irradiation in *Anethum graveolens* (Fig. 4). In addition, both *P. crispum* and *Anethum graveolens*, emitted in low amounts longicyclene, a stress induced sesquiterpene, under WLAN-frequency irradiation.

CONCLUSIONS

The presented data collectively suggest that microwave irradiation constitute a stress to the plants, resulting in enhanced emissions of green leaf volatiles, up-regulation of terpenoid emissions and modification in essential oil content and foliage anatomy. Anatomical and emission traits suggested that WLAN-frequency irradiation resulted in more severe stress than GSM-frequency irradiation, but the effect of WLAN-frequency irradiation on essential oil was inhibitory. There was an agreement between anatomical and chemical traits with anatomically most resistant species *Apium graveolens* being chemically least responsive.

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Abbreviations

VOC	volatile organic compounds
GLV	green leaf volatiles
WLAN	wireless router
GSM	mobile devices
TEM	transmission electron microscopy

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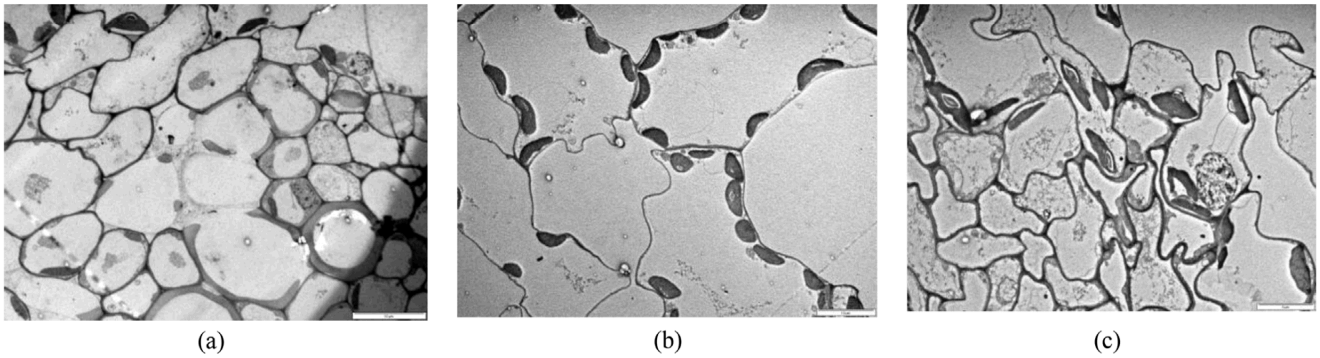
(a) (b) (c)

Fig. 1.

TEM images of cell walls in leaves of microwave-irradiated and control parsley: a) Control; b) GSM irradiated; c) WLAN iradiated.

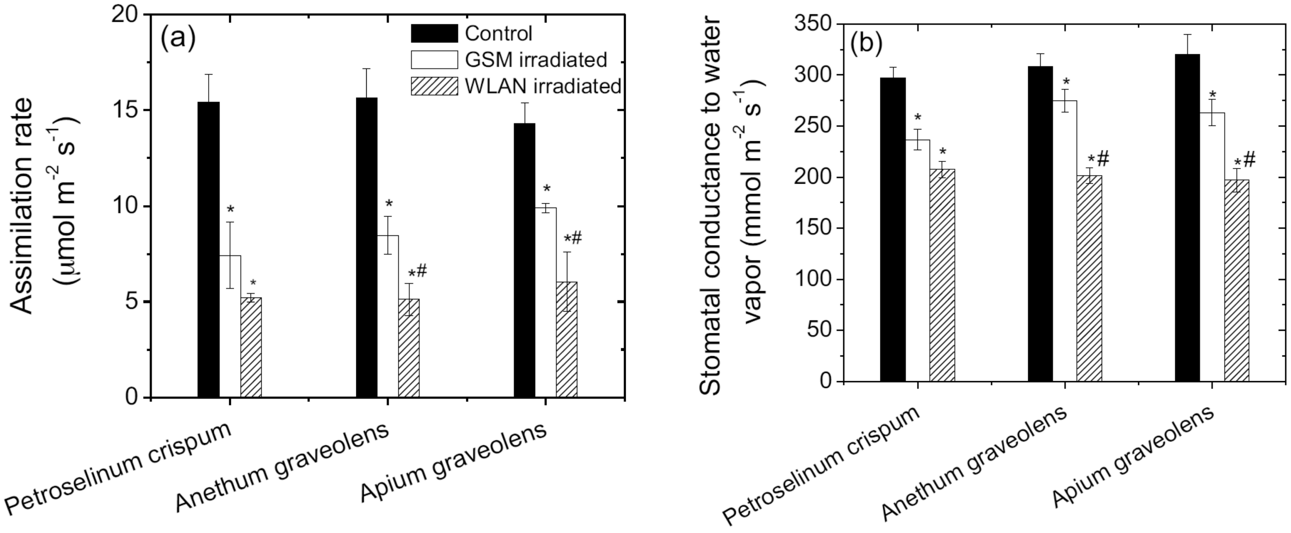

Fig. 2.

Changes in net assimilation rate (a) and stomatal conductance to water vapour (b) in 3 aromatic plants in response to microwave stress. The data are expressed per unit projected leaf area. Each data point is the mean (± SE) of 8 independent replicate experiments with a different plant.

* and # demonstrates statistically significant differences between the microwave irradiated plants and control plants and between WLAN and GSM irradiated plants respectively ($P < 0.05$).

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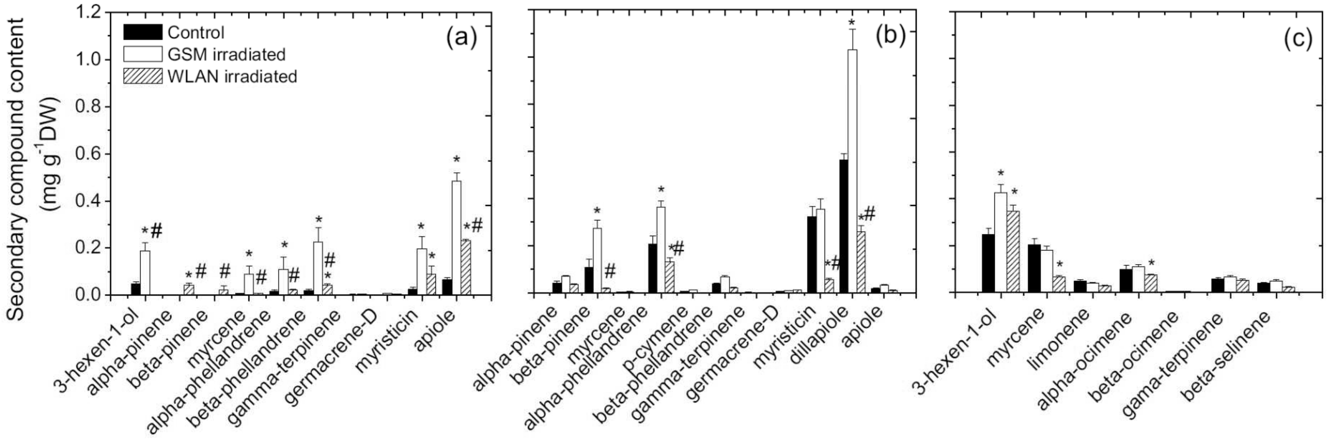

Fig. 3.

Changes in terpene content (mg g^{-1} FW) in *Petroselinum crispum* (a), *Anethum graveolens subsp. hortorum* (b) and *Apium graveolens* (c) foliage in response to microwave irradiations at bands corresponding to wireless router (WLAN) and mobile devices (GSM). Each data point is the mean (± SE) of three independent replicate experiments with a different plant. * and $^{\#}$ demonstrates statistically significant differences between the microwave irradiated plants and control plants and between WLAN and GSM irradiated plants respectively ($P < 0.05$).

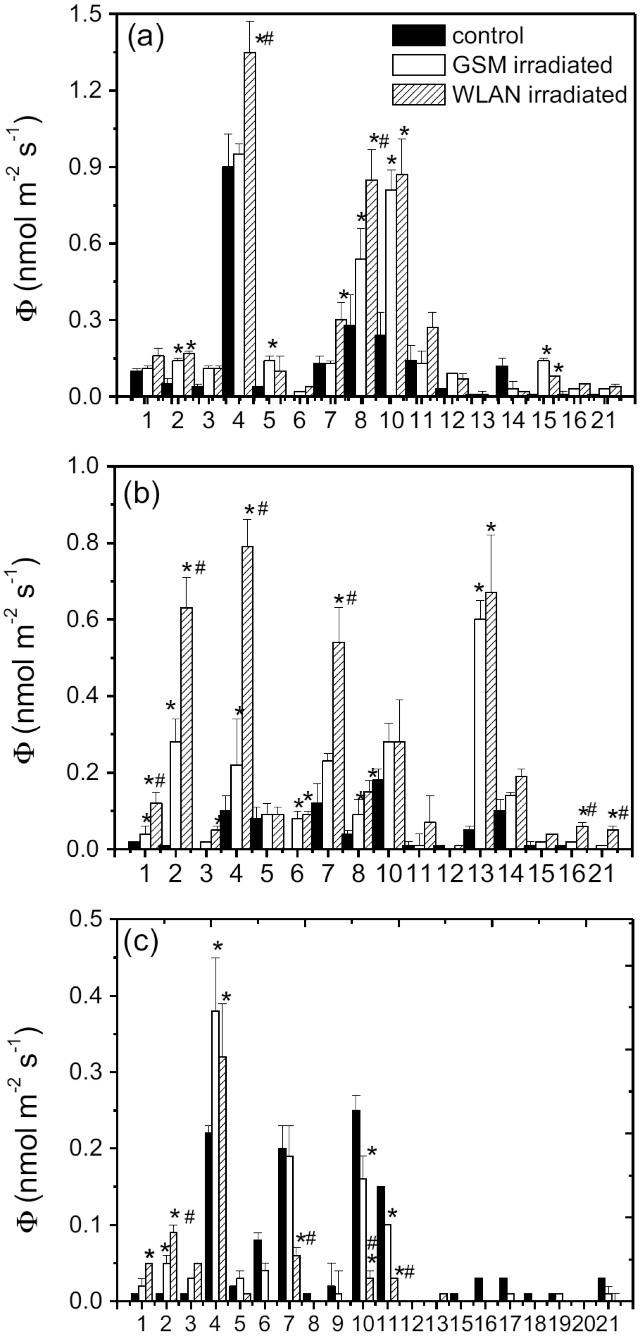

Fig. 4.

Alteration of the emission of volatile organic compounds (nmol m^{-2} s^{-1}) from foliage of *Petroselinum crispum* (a), *Anethum graveolens subsp. hortorum* (b) and *Apium graveolens* (c) in response to microwave irradiations at bands corresponding to wireless router (WLAN) and mobile devices (GSM) (presentation of statistical differences as shown in Fig. 2). Each number corresponds to a particular volatile compound as follows: **1.** 1-hexanol; **2.** *(Z)*-3-hexen-1-ol; **3.** (*E*)-2-hexenal; **4.** α-pinene; **5.** camphene; **6.** β-myrcene; **7.** β-pinene; **8.** α-phellandrene; **9.** Δ-3-carene; **10.** D-limonene; **11.** *para*-cymene; **12.** β-phellandrene; **13.** (*E*)-

β-ocimene; **14.** 1,8-cineol; **15.** iso-bornyl acetate; **16.** longicyclene; **17.** caryophyllene oxide; **18.** α-selinene; **19.** (*Z*)-β-farnesene; **20.** α-caryophyllene; **21.** geranylacetone.

* and # demonstrates statistically significant differences between the microwave irradiated plants and control plants and between WLAN and GSM irradiated plants respectively ($P < 0.05$).

Table 1

Ultrastructural analysis of the leaves of studied plants. The average values (± SE) are replicates of six independent measurements with different plants.

Treatment	Cell wall thickness (μm)	Chloroplast length (μm)	Chloroplast area (μm^2)	Mitochondrion length (μm)	Ratio of starch grain area to chloroplast area (%)
			Petroselinum crispum		
Control	0.300 ± 0.07	6.78 ± 0.12	13.31 ± 0.22	1.00 ± 0.27	8.93 ± 0.13
860 – 910 MHz	0.250 ± 0.06	6.76 ± 0.28	8.491 ± 0.06	0.90 ± 0.13	9.99 ± 0.12
2.4 - 2.5 GHz	0.200 ± 0.05	6.50 ± 0.16	7.807 ± 0.11	0.70 ± 0.05	6.01 ± 0.08
			Anethum graveolens		
Control	0.187 ± 0.01	5.90 ± 0.13	8.43 ± 0.23	1.68 ± 0.13	5.21 ± 0.13
860 – 910 MHz	0.175 ± 0.01	5.20 ± 0.17	8.08 ± 0.29	1.00 ± 0.25	8.13 ± 0.08
2.4 - 2.5 GHz	0.175 ± 0.01	4.85 ± 0.31	7.04 ± 0.22	0.80 ± 0.10	0
			Apium graveolens		
Control	0.160 ± 0.01	5.80 ± 0.20	7.68 ± 0.14	1.57 ± 0.08	5.34 ± 0.28
860 – 910 MHz	0.156 ± 0.01	4.33 ± 0.26	7.06 ± 0.35a	0.55 ± 0.13	0
2.4 - 2.5 GHz	0.136 ± 0.01	3.33 ± 0.26	6.68 ± 0.14	0.25 ± 0.13	0